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14. ABSTRACT
It is fundamentally important to understand the underlying mechanisms regulating prostate caner (PCa) metastasis. Despite the increased PSMA expression found in more advanced stage of PC a, little is known about the functional role of PSMA in PCa progression. Work accomplished for the period of the report has (1) generated the fluorescently labeled anti-PSMA antibodies for monitoring PSMA expressions in live PCa cells, (2) established the cell model systems with reduced PSMA expression for studying PSMA functions, (3) identified fibronetin as a specific extracellular matrix for enhanced LNCaP attachment and (4) performed 2-D wound healing assays to examine the role of PSMA in PCa cell migration. Results from these studies demonstrated and further supported the idea that PSMA is involved in PCa cell adhesion and migration, therefore will enhance our understanding of molecular regulatory mechanisms of PCa. Knowledge about the action of PSMA in cell adhesion and migration during PCa metastasis will have a direct impact on the improvement for design better-targeted approaches for treating patients suffering from metastatic prostate cancer.

15. SUBJECT TERMS
prostate cancer, prostate-specific membrane antigen (PSMA), cell adhesion, cell motility andmigration, anti-PSMA antibody, prostate cancer metastasis

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Table of Contents

Page

INTRODUCTION

PCa metastasis is a multi-step process that involves a variety of molecules affecting the severity of the disease and the response to treatments (1-3). Prostate-specific membrane antigen (PSMA) has been recognized as the single most well-established and highly restricted membrane antigen for PCa and its expression is upregulated approximately 10-fold in advanced stages of metastasis and in hormone-refractory PCa (4-13). These observations indicate the importance of PSMA in PCa and its application in targeted clinical treatments. Little is known about the functional role of PSMA in PCa progression. Previously, we demonstrated that expression of PSMA resulted in morphologically better-spreading cells with redistributed F-actin and beta-1 integrin. Those molecules are known to play key roles in cell adhesion and motility. Furthermore, we observed motility changes in cells with their PSMA enzymatic activity inhibited by specific anti-PSMA antibody. These findings suggest a potential role of PSMA in adhesion and migration, the critical processes during PCa metastasis. Currently, there is no effective treatment for advanced PCa. This proposed study is aimed to establish the role of PSMA and its enzymatic activity in PCa cell adhesion and migration which can be used to develop novel strategies for diagnosis and therapeutic treatment for human metastatic PCa.

BODY

1. Statement of Work (SOW)

Task 1. To investigate the effect of PSMA expression on PCa cell adhesion

Anti-PSMA antibody will be conjugated directly with fluorescent probes for live cell imaging. PSMA distribution of cells grown on different extracellular matrices will be characterized to provide guidance for designing the motility assays. Assays measuring cell attachment and detachment will be employed for the identification of a functional role of PSMA in cell adhesion.

Task 2. To characterize the effect of PSMA expression on PCa cell motility and migration

Experiments are designed to test whether increased expression of PSMA in higher grade cancer is the result of its role in regulating cell motility and migration during PCa metastasis. Changes in cell motility and migration will be measured by *in vitro* 2D and 3D migration assays. Results from these studies will directly indicate the function of PSMA in the particular stages during PCa progression.

Task 3. To determine whether the enzymatic activity of PSMA directly regulates the adhesion and migration of PCa cells

Cell attachment, detachment and migration assays will be performed in the presence or absence of J415, a specific anti-PSMA antibody with measurable inhibitory effect on PSMA enzymatic activity. In addition, a possible link between PSMA enzymatic activity and integrin activity and function will be examined using J415 and P4C10, a functional inhibitory anti-integrin antibody. Demonstrating a connection between PSMA enzymatic activity and integrin activity will advance our understanding of the regulatory machinery controlling PCa metastasis.

2. Study and Results

Since the start of the award and particular in the past 12 months, we have concentrated our efforts towards the achievement of proposed specific tasks of the approved Statement of Work (SOW). Significant progress has been made for the project. Two manuscripts are under preparation. In the following, we described and summarized our research findings for the first 12 months and the past 12 months.

Most of the work proposed in task1 was achieved in the first 12 months of the funding.

Task 1. To investigate the effect of PSMA expression on PCa cell adhesion

1a. Generate fluorescent-conjugated antibodies for live cell adhesion and motility assays

Conjugating fluorescent dyes directly to an antibody has been demonstrated to be a powerful tool for investigating protein functions (14-15). We successfully generated several photo-stable fluorescent probes Alexa488, Alexa546 and Alexa633-conjugated anti-PSMA mAbs J591 and J415 which directly against extracellular epitopes of PSMA (16). We examined the binding characteristics of these antibodies after the conjugation and found that conjugating the antibody with fluorescent probes did not alter their binding properties. These fluorescent-conjugated antibodies have been used to monitor PSMA expression and for the examination of its functional role in cell adhesion (shown in Figures of this report). They will also be used for future studies to establish the role of PSMA in cell migration and its association with integrin in live PCa cells.

1b. Optimize PSMA siRNA transfection in LNCaP cells to reduce PSMA expression

One of the most important accomplishments during the first 12 months funding is our ability to significantly increase the efficiency of transfection with siRNA of PSMA. We established a protocol that, with transient transfection of siRNA of PSMA, we could achieve an approximately 90% reduction of PSMA expression in LNCaP (shown in Fig. 1A-C in Appendices and Supporting Data section). Furthermore, we were able to further develop a conditional medium to allow LNCaP cell to maintain the reduced PSMA expression for up to 2-weeks. Achieving a significant reduction of PSMA expression and especially maintaining PSMA expression at a reduced level (see in Fig. 1A-B in Appendices and Supporting Data section) will enable us to perform the critical functional studies that otherwise won't be possible. We found that although various factors were critical for the improvement of siRNA transfection, the type of lipid carrier used for the transfection made the biggest difference for the increased transfection rate. Dharmafect3 (Dharmacon) was used to reach the highest level of inhibition of PSMA expression.

1c. Examine the characteristics of PCa cells with varied PSMA expressions grown on different extracellular matrices

To examine whether PSMA plays a role in cell attachment and motility, we tested cell adhesion property of LNCaP cells and PC3 cells on dishes coated with a range of different substrate materials including: uncoated regular glass, coverslips coated with poly-D-lysine, BSA, ECM, fibronectin (FN) and collagen. PCa cell adhesion properties were characterized first by

examining the morphology of cells and the number of cells plated onto various matrix materials. Cell spreading assay (20) was an approach previously used to examine the extent of cell adhesion. We found that LNCaP attachment was greatly enhanced in dishes coated with FN but not uncoated dishes or dishes coated with either BSA or ECM (shown in Fig. 2 in the Appendices and Supporting Data section). It is evident that, shortly after plating, most of the LNCaP cells were only firmly attached onto the FN-coated dishes with well-spread morphology but not onto other extracellular matrices coated dishes. No significant differences in cell adhesion were observed in PC3 cells plated on dishes coated with any of the above extracellular matrices. Our findings suggest a potential interaction between PSMA and integrin, since it is known that fibronectin binds to integrin to promote various cellular functions including cancer progression. We are still working on experimental details to achieve a consistent, even and thin layer of collagen coating on the dishes for conducting adhesion and motility assays.

1d. Perform live cell assays to characterize the relationship between PSMA expression and cell attachment and detachment

To examine whether PSMA expression directly regulate cell adhesion, therefore to modulate PCa migration, using *in vitro* cell model systems and live-cell imaging methods, we characterized the role of PSMA in cell motility and adhesion. Using wound-healing assay, we monitored and recorded live cells motility of PC3 and PC3-PSMA and observed a significant decrease in the distance of cell migration in PC3 cells expressing PSMA (see Fig. 3-4 in the Appendices and Supporting Data section). We found that reduction of PSMA expression in LNCaP cells increased cell motility. Together, these observations provide evidence for the role of PSMA in PCa cell migration. 22Rv-1 cells, a cell line with inherent fluctuation in PSMA expression were used to test PSMA expression in relationship with cell motility. Without any manipulation of the cells (such as transfection), we observed redistribution of F-actin only in 22Rv1 cells expressing high levels of PSMA (see Fig. 5 in the Appendices and Supporting Data section). This result is consistent with our previous finding of altered F-actin in PC3 cells tranfected with PSMA. These data suggest that PSMA may involved in cell motility by regulating the actin cytoskeleton network.

In the past 12 months, our efforts were focused on investigating the effect of PSMA expression in relationship with cell adhesion and motility, as proposed in Task 2 of SOW. Here, we summarized our results (shown Figure 6-10 in the Appendices and Supporting Data section).

Task 2. To characterize the effect of PSMA expression on PCa cell motility and migration

2a. Monitor and characterize the motility of live PCa cells with varied PSMA expressions

Based on our finding that LNCaP cells attached better on FN coated dishes, we began to investigate the molecular mechanism regulating PSMA-positive cell adhesion. Using confocal microscopy, we examined the expression level of PSMA with that of integrin alpha5beta1 and we found that the expression level of PSMA correlated tightly with the level of integrin alpha5beta1 (Figure 6 in the Appendices and Supporting Data section). Moreover, using a functional blocking anti-integrin beta antibody, we have tested its effect on cell attachment. As shown in Figure 7 in the Appendices and Supporting Data section, reduction of attachment was observed in PSMA-positive LNCaP cells but not in PSMA-negative PC3 cells. Together, these

observations indicated the potential interaction of PSMA with integrin-related signaling pathways to regulate cell motility and adhesion.

2b Characterize the 2D-migration of PCa cells with varied PSMA expressions using wound healing model systems

To tested if the expression of PSMA is sufficient to change the properties of PSMA-positive cell motility, we used LNCaP-siRNA-PSMA cells with reduced PSMA expression. We tested their motility in a wound-healing assay while comparing it with control LNCaP cells. As shown in Fig. 8, reduction of PSMA expression by siRNA increased LNCaP cell motility. We discovered that significantly higher numbers of cells with lower PSMA expression were migrated into the wounded area. Interestingly, cells with reduced level of PSMA also migrated for longer distance when compared with that of the control untransfected LNCaP cells with higher PSMA levels. Further investigation with different culture conditions provided additional evidences for this negative correlation of PSMA level with cell motility (shown in Fig. 9 in the Appendices and Supporting Data section). These findings demonstrate the involvement of PSMA in cell motility and adhesion.

2c. Examine the efficiency of transmigration as a function of PSMA expressions using 3D matrigel model system

To test the relationship between PSMA expression and cell motility, we discovered that transfection of siRNA-PSMA using lipofectatmine resulted in relatively low transfection efficiency and produced LNCaP cell colonies (3D) with mixed population of LNCaP cells at various levels of PSMA expressions. We found that, at the edge of the expanding colonies, the majority of the cells that moving outward were the cells with reduced PSMA levels. On the contrary, at the centers (slow motility) were cells with high levels of PSMA (Figure 10 in the Appendices and Supporting Data section). These data indicated that PSMA play a role in regulating cell adhesion but not in promoting cell motility. We plan to further confirm these results using 3D matrigel model system to examine the PSMA function in cell migration.

Task 3. To determine whether the enzymatic activity of PSMA directly regulates the adhesion and migration of PCa cells

We have started to investigate if PSMA enzymatic activity is involved in Pca cell adhesion. We found that pretreatment of LNCaP cells with PSMA enzymatic inhibitory antibody J415 resulted in reduced attachment of these cells (shown in Figure 11 in the Appendices and Supporting Data section). These data suggest that PSMA enzymatic activity may be involved in regulating PSMA-positive Pca cell adhesion.

Currently, our research is focused on further characterizing the interaction of PSMA expression on cell motility and adhesion. Study is ongoing to determine whether PSMA enzymatic activity plays a role in cell adhesion and migration. Since this project has been carried out according to our proposed timeline, we do not expect to have major alterations to our proposed research plans.

KEY RESEARCH ACCOMPLISHMENTS:

For the first 12 months

1. Generated fluorescently conjugated anti-PSMA antibodies for live cell imaging.

2. Optimized the siRNA-PSMA transfection and achieved an approximately 90% reduction of PSMA expression in LNCaP cells.

3. Developed a conditional medium for LNCaP cell to maintain the reduced PSMA expression.

4. Found enhanced LNCaP cell attachment onto fibronectin-coated dishes.

5. Expression of PSMA in PSMA negative PC3 cells reduces cell motility.

For the past 12 months

6. Reduced expression of PSMA correlates with decreased levels of integrin alpha5-beta1.

7. Anti-integrin antibody specifically inhibited the attachment of PSMA-positive LNCaP cells.

8. Increased LNCaP cell motility is associated with reduced PSMA expressions.

9. PSMA enzymatic activity may be involved in cell adhesion.

REPORTABLE OUTCOMES:

Two manuscripts are currently in preparation for publication:

1. Lin SX, Navarro V, Liu H, and Bander, NH. A functional role of PSMA in prostate cancer cell migration. Manuscript in preparation.

2. Lin SX, Navarro V, Liu H, and Bander, NH. PSMA is involved in cell adhesion by interacting with integrin in prostate cancer cells. Manuscript in preparation.

Presentation and Abstract:

1. Lin, SX, Navarro V, Liu H and Bander NH. A Functional Role of Prostate-Specific Membrane Antigen in Prostate Cancer Metastasis. AACR meeting at Washington DC, 2006.

2. Lin, SX, Navarro V, Sae, K, Liu H and Bander NH. Investigating the Functional Role of Prostate-Specific Membrane Antigen and its Enzymatic Activity in Prostate Cancer Metastasis. IMPACT meeting, Atlanta GA, 2007.

CONCLUSION

The goal of the proposal is to investigate the function of PSMA in prostate cancer metastasis. Based on our accomplishment in the past 24 months, results from these studies demonstrated and further supported the idea that PSMA is involved in PCa cell adhesion and migration. Work from this period of the report also generated the critical tools (agents and model systems) for our continuing efforts for the understanding of PSMA function in prostate cancer progression. In the following funding year, we will continue to pursue the tasks as outlined in the approved Statement of Work (SOW). We are confident that our work will provide new knowledge for the understanding of prostate cancer metastasis.

The outcome from our study will have a direct impact on the improvement of treatments including immunotherapy for human PCa. Knowledge about the action of PSMA in specific event during PCa metastasis will help to design better-targeted approaches for treating patients suffering from metastatic prostate cancer. Currently, monoclonal antibodies against PSMA tested in Phase I-II clinical trials have shown great potential. Identifying the functional role of PSMA in cell adhesion-related events will help to provide guidance for choosing the best suited antibody and the most appropriate toxins for conjugation, therefore to maximize the therapeutic efficacy and reduce side effects for human PCa immunotherapeutic treatment.

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APPENDICES and SUPPORTING DATA:

Total of 11 figures are included in this report.

Figure 1. Reduction of PSMA Expression by Transfection with siRNA-PSMA

Figure 2. Attachment of PCa cells on Various Extracellular Matrices

Figure 3. Effect of PSMA Expression on PSMA-Negative PC3 cell Migration

Figure 4. Cell Motility of PC3 and PC3-PSMA Cells

Figure 5. Distribution of F-actin in 22Rv1 Cells with Different PSMA Expression Levels

Following are data obtained in the past 12 months

Figure 6. Reduced PSMA Expression correlates with decreased integrin expression

Figure 7. Effect of Functional Blocking Anti-Integrin Antibody on Cell Attachment

Figure 8. Effect of Reduced PSMA Expression on LNCaP Cell Motility

Figure 9. Effect of Reduced PSMA Expression on LNCaP Cell Motility under Different Culture Conditions

Figure 10. Effect of Reduced PSMA Expression on LNCaP Cell Motility

Figure 11 Effect of Reduced PSMA Enzymatic Activity on Cell Attachment

Figure 1. Reduction of PSMA Expression by Transfection with siRNA-PSMA

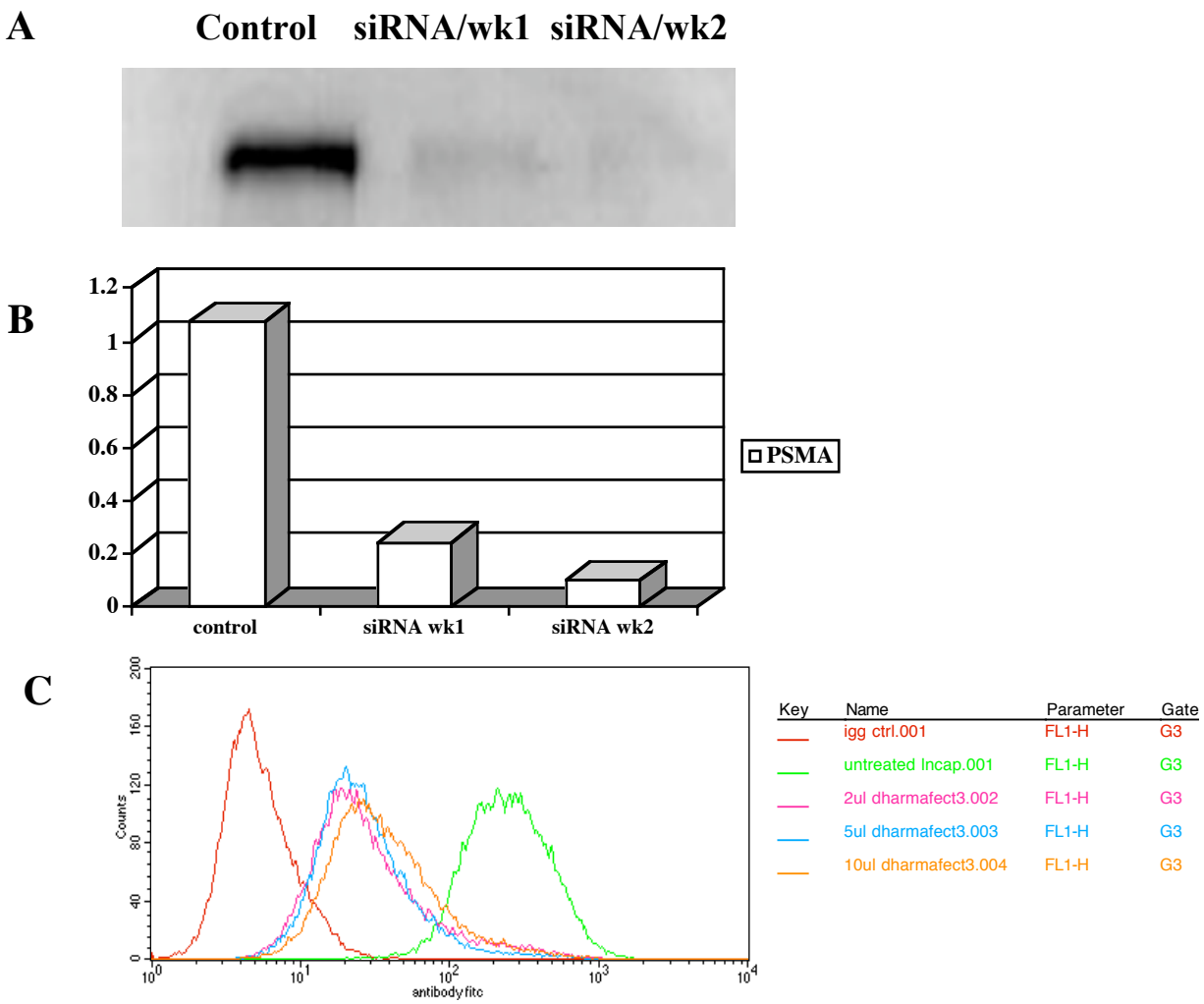


Figure 1. Transfecting siRNA of PSMA to LNCaP cells reduces their PSMA expression. LNCaP cells were transfected with siRNA of PSMA by incubation with a mixture of siRNA and Dharmafect3 for 5 hrs before switching to growth medium containing 1/3 of the transfection mixture for indicated period of times. (A), Cell lysate was collected after siRNA transfection for 1 week or 2 weeks and Western blot analysis was performed to evaluate the extend of PSMA reduction. Cell lysate from untransfected LNCaP cells were used as control for normal PSMA levels in LNCaP cells. (B), the protein levels in A were measured, quantified (The level of PSMA expression was normalized with the actin) and plotted to demonstrate the PSMA levels shown in A. (C), FACS analysis was conducted in cells transfected for 3 days with different amount of Dharmafect3 (2-10ul) and the level of PSMA expression on cells were plotted.

Result: Reduction of PSMA, approximately 90%, was observed in LNCaP cells transfected with siRNA of PSMA. This reduction of PSMA expression lasted for prolonged period of time. Significant reduction of PSMA expression was achieve using a range of Dharmafect3 at 2-10ul/ml mixed with 20nM siRNA-PSMA for transfection.

Figure 2. Attachment of PCa cells on Various Extracellular Matrices

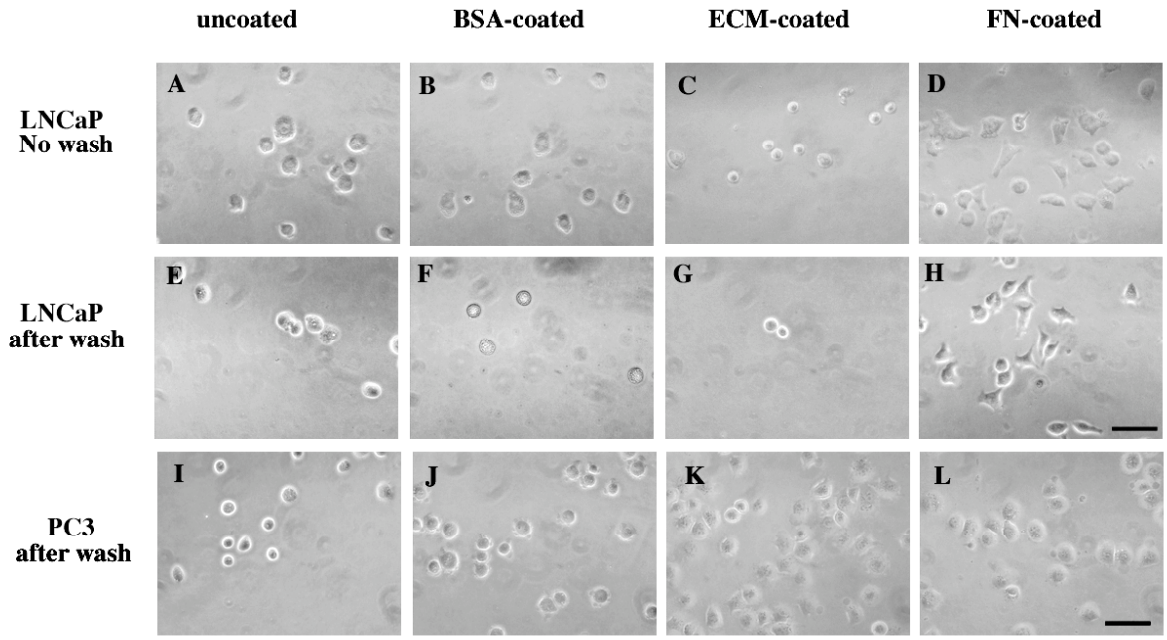


Figure 2. Increased LNCaP adhesion was observed in dishes coated with fibronectin. PSMA-positive LNCaP cells (A-H) and PSMA-negative PC3 cells (I-L) were plated onto triplicates of uncoated dishes (A, E, and I) or dishes coated with 2% BSA (B, F, and J), 10μg/ml extracellular matrix (ECM) (C, G and K) or 10μg/ml fibronectin (FN) (D, H and L). Cells were plated for 60 min without wash (A-D) or washed with serum free RPMI medium for 3 times (E-L). After washes, all the unadherent cells were rinsed away, leaving only the adherent cells for imaging collection. Bar=20μm.

Results: Morphologically better spreading LNCaP cells were onto FN-coated dishes (D) but not of cells plated on uncoated dishes or dishes coated with either BSA or ECM (A-C, respectively). Enhanced cell attachment and higher number of cells were observed on FN-coated dishes after washes (H). Reduced numbers of LNCaP cells were left on uncoated dishes after wash (E). Limited cells could be seen on dishes coated with either BSA or ECM (E-G, respectively). Significant differences observed in cell morphology, attachment and cell number were observed of PSMA-negative PC3 cells plated on any of the matrix materials tested (I-L) with or without washes.

Figure 3. Effect of PSMA Expression on PSMA-Negative PC3 cell Migration

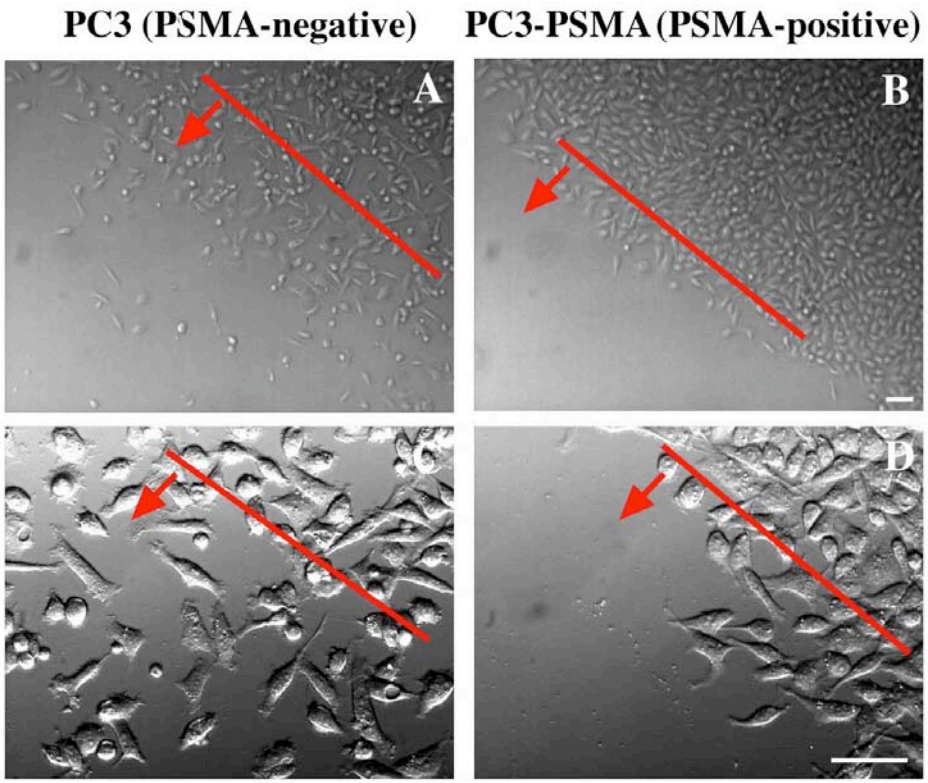


Figure 3. Expression of PSMA in PC3 cells reduces its cell motility.
PC3 and PC3 cells stably transfected with PSMA (PC3-PSMA) were grown to confluent monolayer before wounding. Cells were recovered for 1 hour after wounding in regular growth medium and DIC images were collected after 6 hrs in growth medium (A-D). Images in C and D are higher (3X) magnification comparing to images in A and B. The lines indicate the edges of wounded area and the arrows point to the direction of cell migration. Bar=20uM.

Result: Expression of PSMA in PC3 cells slows cell migration.

Figure 4. Cell Motility of PC3 and PC3-PSMA Cells

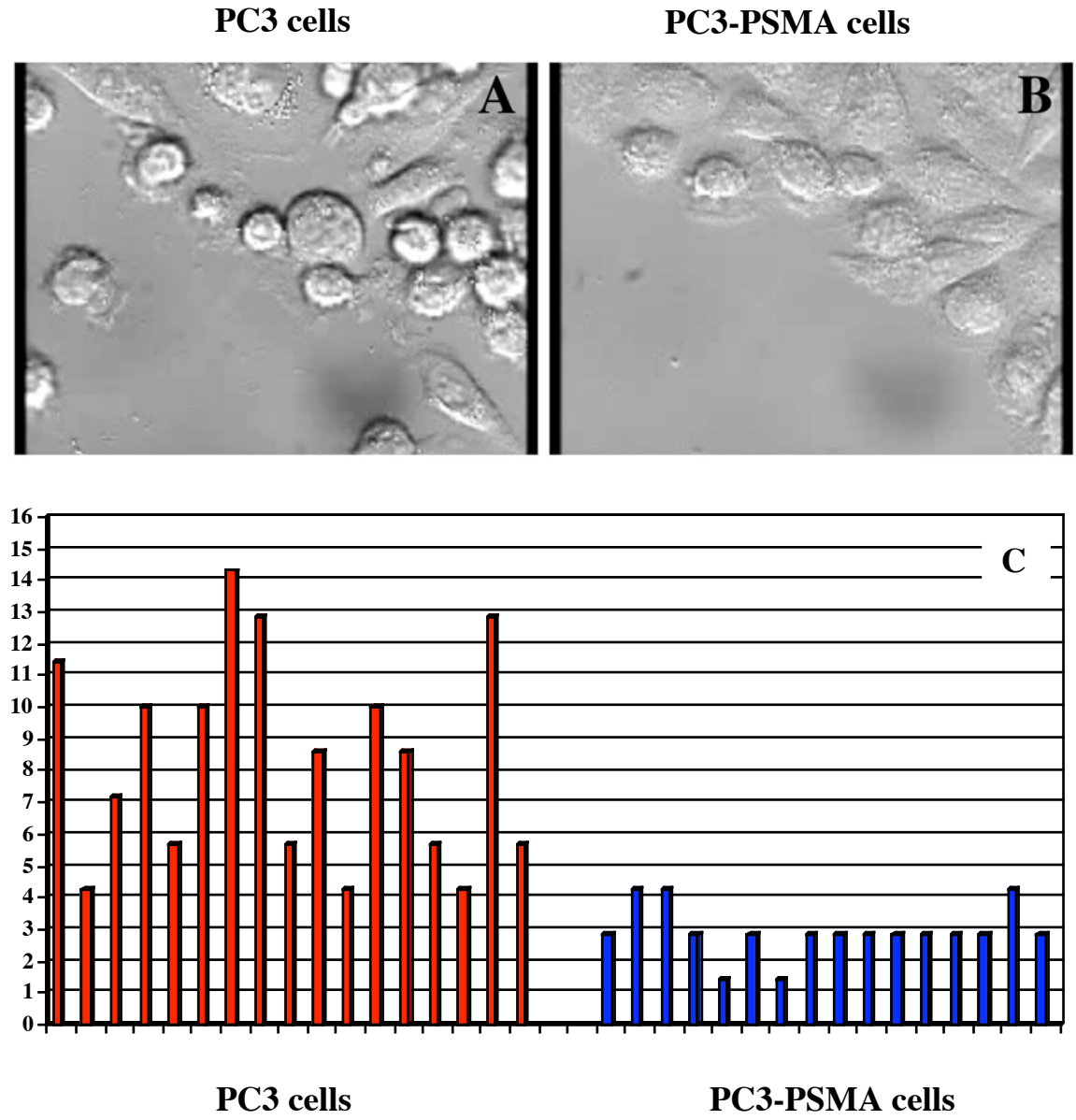


Figure 4. Live cell motility of PC3 and PC3-PSMA cells
Live cell motility of PC3 (A) and PC3-PSMA (B) cells was recorded after wounding. Total recording time=30min. In C, the distances of cell migration of PC3 (n=17) and PC3-PSMA (n=16) shown in Fig. 4A-B were measured on the migratory tracks produced by individual PC3 and PC3-PSMA cells shown in A-B. The X-axis indicates the individual cells and the Y-axis showes the values of the relative distances the cell migrated during the recorded time.

Result: PSMA expression reduces PC3 cell motility.

Figure 5. Distribution of F-actin in 22Rv1 Cells with Different PSMA Expression Levels

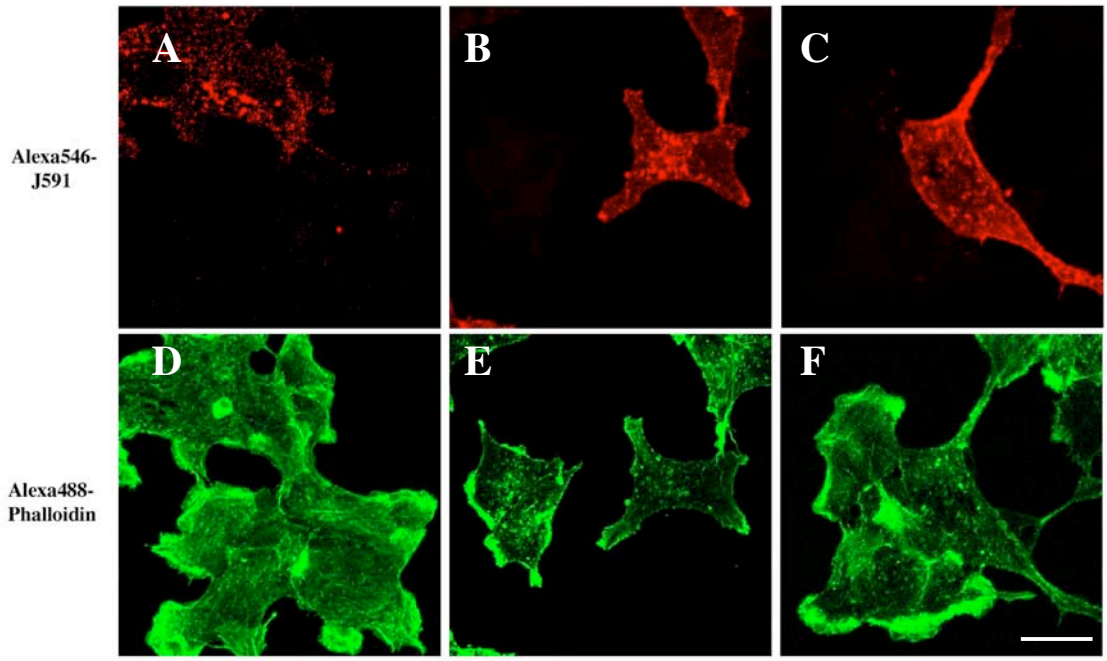


Figure 5. Distribution of PSMA and F-actin in 22Rv1 cell
22Rv1 cells fixed in 3.3% paraformaldehyde and permeabilized with 0.025% saponin were stained with Alex546-J591 (A-C) and Alexa488-phalloidin (D-F). Projections of confocal images from three different fields (A&D, B& E and C&F, respectively) of the same experiment are shown. Bar=10um.

Results: Different levels of PSMA expression were observed in 22Rv1 cells (inherited characteristics of this cell line without any manipulations) and alteration in F-actin organization was observed only in 22Rv1 cells with higher levels of PSMA expression**,** indicating the potential interaction of PSMA with signaling pathway in regulating cell adhesion and migration.

Data shown in Fig. 6-11 are completed during the last 12 months

Figure 6. Effect of Reduced PSMA Expression on Integrin Levels

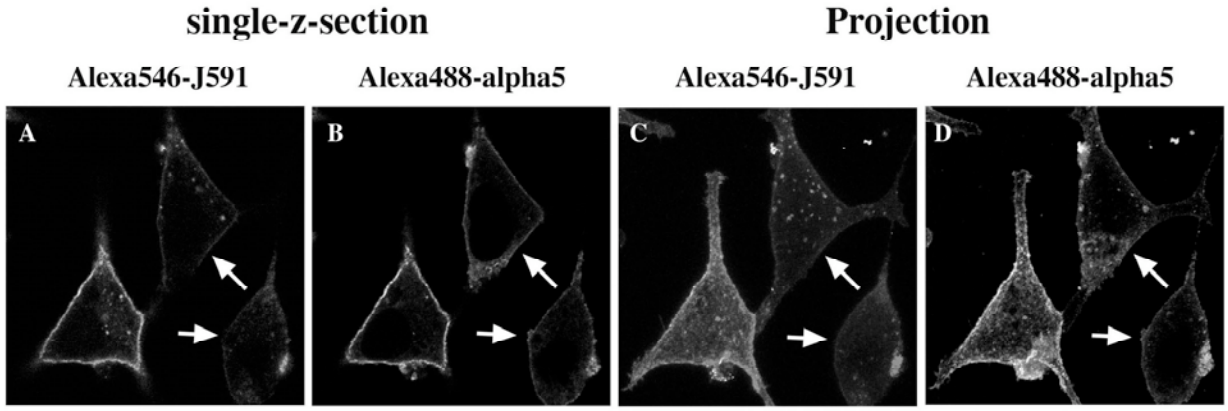


Figure 6. Reduction of PSMA expression correlates with decreased ingetrin level in LNCaP cells.
LNCaP cells (A-D) transfected with siRNA-PSMA were fixed and labeled with fluorescence conjugated monoclonal anti-PSMA antibody Alexa546-J591 and polyclonal alpha5 integrin antibody (Alexa488). Confocal images were collected after labeling. Single sections (A-B) and projected images (C-D) were shown. Arrows pointed to the siRNA-PSMA transfected cells.

Result: Reduction of PSMA expression by siRNA correlates with integrin alpha5-beta1 levels in LNCaP cell. In cells with reduced PSMA expressions (indicated by arrows), a decreased level of integrin alpha5 beta1 was also observed.

Figure 7. Effect of Functional Blocking Anti-integrin Antibody on Pca Cell Attachment

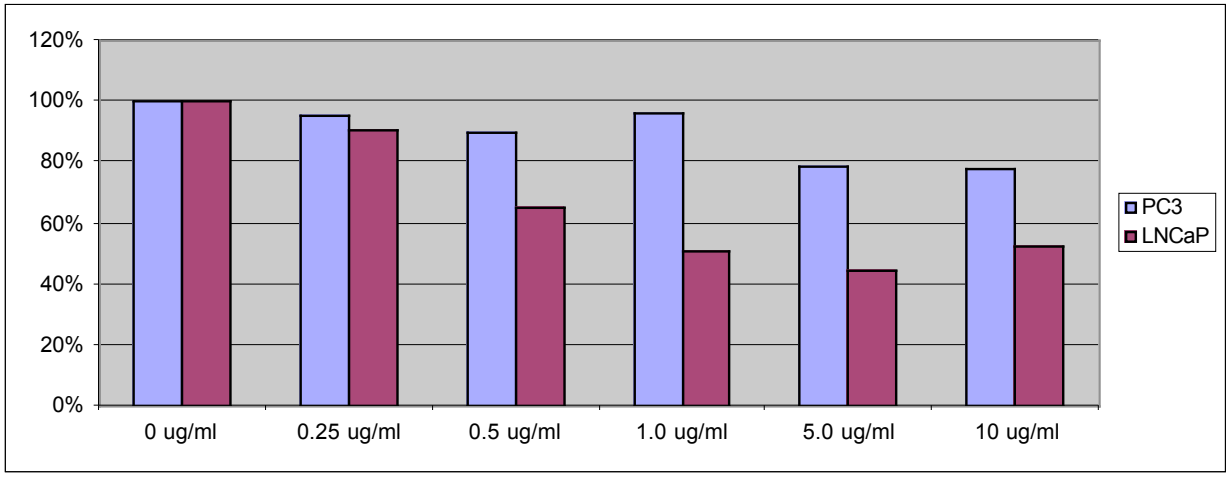


Figure 7. Cell attachment assay with functional blocking anti-integrin antibody
LNCaP cells and PC3 cells were treated with 0-10ug/ml functional blocking anti-integrin antibody before for 15 min before plating onto FN coated coverslips for 60 min. After n attached cells were washed out, Numbers and the percentage of cells attached on the dishes were counted and compared with the control untreated cells (set to be 100%).

Result: Significant reduction of cell attachment was observed with PSMA-positive LNCaP cells but not with PSMA-negative PC3 cells when treated with functional blocking anti-integrin antibody. Such an effect was shown to be antibody concentration dependent.

Figure 8. Effect of Reduced PSMA Expression on LNCaP Cell Motility

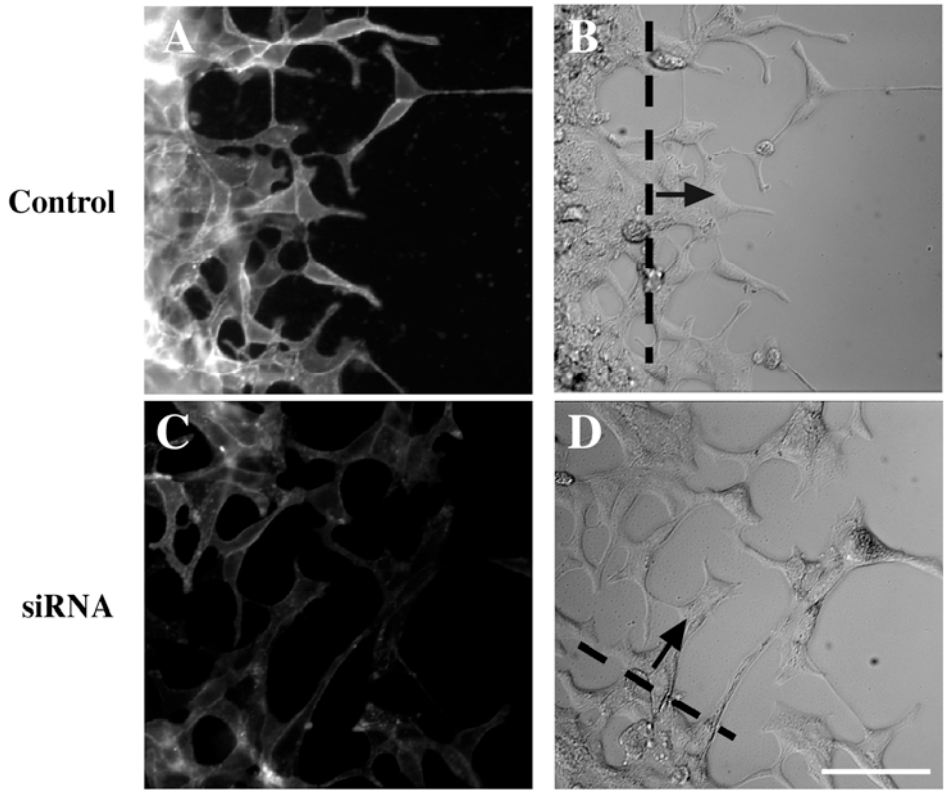
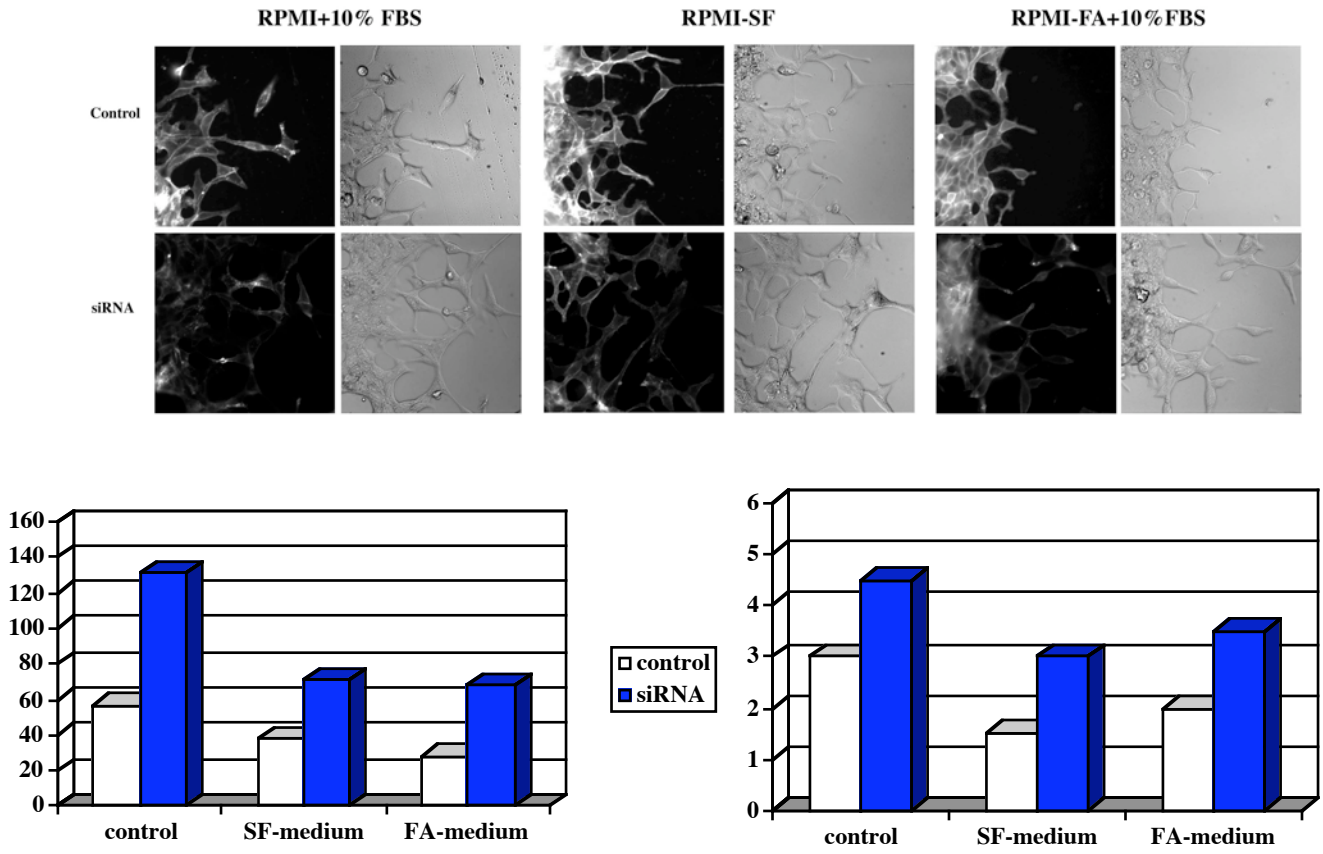


Figure 8. Reduction of PSMA expression increases PSMA-positive LNCaP cell motility. Control LNCaP cells (A-B) and LNCaP cells transfected with siRNA-PSMA (C-D) were grown until confluence before wounding. After wounding, cells were recovered for 1 hour after wounding in regular growth medium and were grown in medium without serum for 48 hr. DIC images were collected after 48 hrs in growth medium without serum (A-D). The dotted lines indicate the edges of wounded area and the arrows point to the direction of cell migration. Bar=20uM.

Result: Reduction of PSMA expression by siRNA increase LNCaP cell motility. Significantly higher numbers of cells with low PSMA expression (C-D) was migrated into the wounded area. Interestingly, these transfected cells (C-D) also migrated for longer distance when compared with the control untransfected LNCaP cells that had higher PSMA levels (A-B)

Figure 9. Effect of Reduced PSMA Expression on LNCaP Cell Motility under Different Culture Conditions



Number of cells migrate

Distance of cells migrated

<u>Figure 9. Reduction of PSMA expression increases PSMA-positive LNCaP cell motility under different culture conditions</u>

Control LNCaP cells or LNCaP cells transfected with siRNA-PSMA were grown until confluence before wounding. After wounding, cells were recovered for 1 hour after wounding in regular growth medium and were grown in medium without serum for 48 hr. Cells were than incubated in the different culture medium (with 10% FBS, serum free medium or medium without folate) as indicated above. After 48 hours, the number of cells and the distance of cells migrated were measured and plotted.

<u>Result:</u> Significant increase of cell migration was observed in siRNA PSMA transfected cells under all the medium conditions when compared with that of control non-transfected, indicating PSMA functions in cell adhesion.

Figure 10. Effect of Reduced PSMA Expression on LNCaP Cell Motility

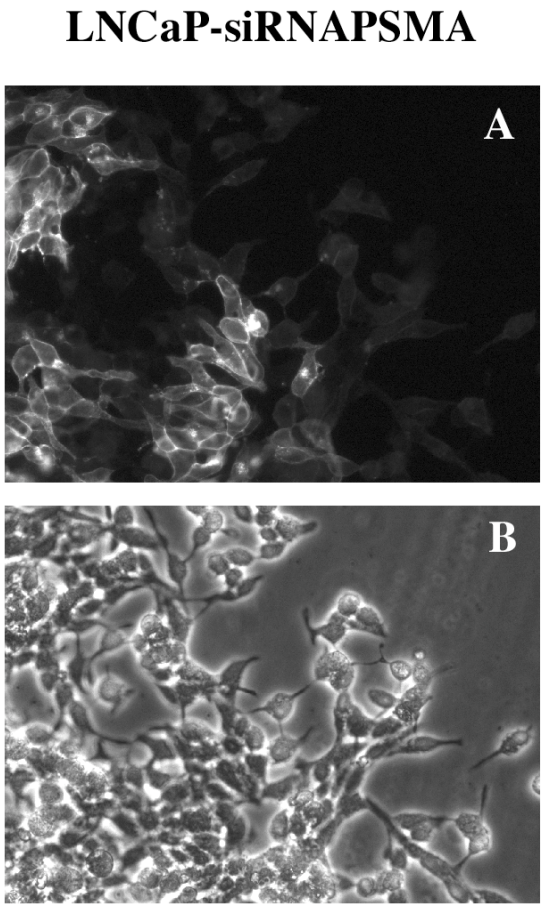


Figure 10. Reduction of PSMA expression increases PSMA-positive LNCaP cell motility
LNCaP cells transfected (lipofectatmine) with siRNA-PSMA were plated onto dishes with high local concentrations of cells for colony formation. During the expansion of colonies, some cells started to move outward and appeared at the expanding "edges" of the colonies. Fluorescent images showing PSMA expressions with Alexa 546 conjugated J591 (A) and DIC images (B) of the cells were taken at edge of the colonies.

Result: Transfection of siRNA-PSMA with Lipofectamine was less effective and resulted in a low percentage of LNCaP cells with reduced levels of PSMA expression. We observed that, at the edge of the colonies, the majority of the cells that moving outward were the cells with reduced PSMA levels. On the contrary, cells at the centers (slow motility) were with high levels of PSMA expression. These data indicated that PSMA may involved in regulating cell adhesion but not in promoting cell motility.

Figure 11 Effect of Reduced PSMA Enzymatic Activity on Cell Attachment

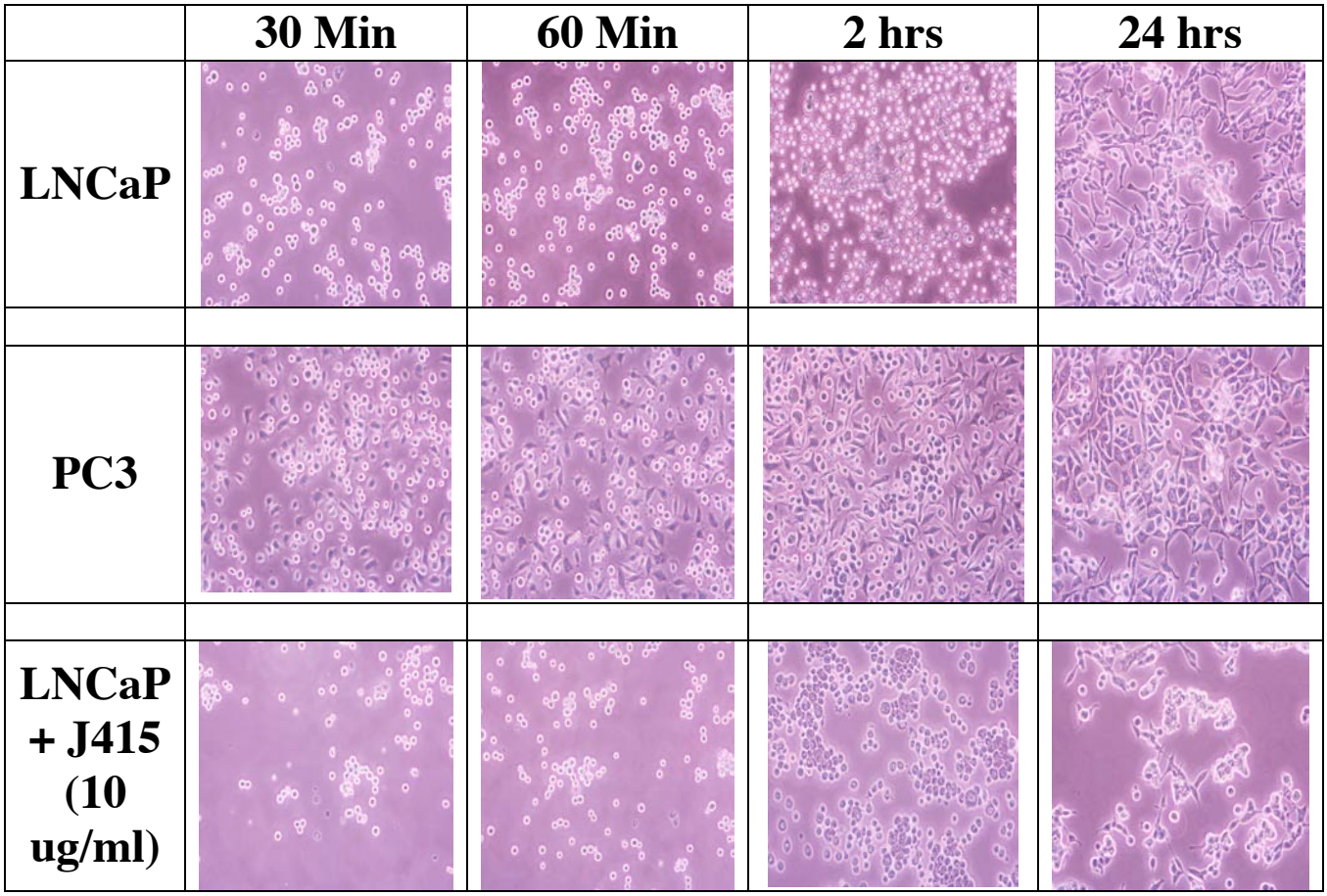


Figure 11. Reduction of PSMA enzymatic activity by anti-PSMA inhibitory antibody J415 decreases PSMA-positive LNCaP cell attachment

Equal numbers of LNCaP cells, PC3 cells and LNCaP cells treated with PSMA enzymatic inhibitory antibody J415 at 10ug/ml for 15min before plating were allowed to attach onto dishes for 30min, 60min, 2 hr and 24hrs as indicated. After removal of unattached cells by rinsing the dishes with medium, the attached cells were counted and images were collected. A representative field for each condition indicated was shown.

Result: Significantly reduced numbers of LNCaP cells incubated with J415 were attached onto dishes at all the time points when compared with that of control LNCaP cells. In addition, it appeared that LNCaP cells treated with J415 also displayed a less adherent and more rounded morphology when compared with that of control LNCaP cells. These data suggest that PSMA enzymatic activity may be involved in regulating PSMA-positive Pca cell adhesion.